[Dep]artment of Agriculture Agricultural Research Service

July 1990

Agricultural Research

Has the Fire Ant Met Its Match?

Modern Rebel Yell: Yikes, Fire Ants!

A friend of ours thinks that the song "Dixie" should contain a cautionary verse about imported fire ants. While on a fishing trip to Alabama, he crouched down beside an earthen mound—and quickly got to the seat of the matter.

The initial pain was only the first stage of his stinging lesson. Next, the ants' potent alkali venom raised up a powerful itch, and worse. Hours later, nursing tiny raised bumps filled with pus, our hardy outdoorsman found himself pining for his old fishing holes back home. In Massachusetts.

Not only do imported fire ants test the mettle of the hardiest nature lovers, but they're an agricultural pest. As one disappointed gardener lamented, the ant "is destructive to plants, attacking young cabbage, collards, eggplant, and many other plants." He reported that the voracious insect also girdled young citrus trees, destroyed strawberries, and "chewed off practically all the potato vines in the garden."

Mounds in which the ants live are hard little fortresses that spoil otherwise-productive pastures for hay and grazing. In crop fields, the earthen ant abodes have torn the blades off many a harvesting machine. These mounds are murder on grassmowing equipment as well, according to highway maintenance crews.

Throughout the South, people have grudgingly surrendered ground to the imported fire ant—territory exceeding the conquests of that previous "scourge of the South," General Sherman. In the ants' wake, playgrounds and picnic areas lie abandoned, parks disfigured, golf courses churned up, and cemeteries damaged.

No one who is hypersensitive to stinging insects should risk being stung. In a few tragic cases, including one of a child pushed by the family dog onto an ant mound, the sting of the fire ant has proven fatal. The imported fire ant problem dates back to the early 20th century, when these South American natives were first sighted in the United States near Mobile, Alabama. Once established, though, the insects commenced marching through Georgia—and other neighboring states—on the double.

By 1957, fire ants were occupying about 27 million acres of land; by 1985, it was 230 million acres—an eightfold increase. Today, the ant is at home in every state in the South, from Texas to Virginia. Puerto Rico is also infested.

Will they spread farther north? For many years, scientists believed the ants' limited tolerance for cold would contain their spread. After all, in their native Argentina, fire ants don't range south beyond 38° latitude, which is analogous to the ant's northernmost reaches in the United States today. But recently, concern has mounted that hybridization between red and black varieties may produce a more cold-tolerant fire ant.

The western states' luck may be running out; isolated colonies of the ants have been spotted in California and Arizona. "Eventual establishment of the ants in California and Arizona appears inevitable," warns the December 1989 issue of Nursery Digest. "Once established in California, they will likely infest much of the western seaboard."

Wherever they set up housekeeping, imported fire ants resist efforts to control them. Ask the scientists at the U.S. Department of Agriculture, who for years have been locked in battle against the fire ants, both the red and black forms. From 1957 to 1977, millions of acres were treated with the insecticide Mirex, but without enduring avail.

We now know that widespread application of toxic chemicals may put the environment at more risk than the ants.

Sad to say, even the old household remedy, dousing 'em with boiling water, is virtually ineffective against well-established colonies with multiple queens.

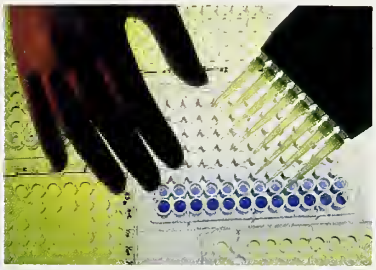
Nevertheless, hope for fire ant control hasn't yet been squashed. This month's cover story, "Turning Up the Heat on Fire Ants" by Jessica Morrison Silva, details the latest anti-ant offensive, featuring a new triad of chemical, biological, and biochemical methods.—By **Regina Wiggen,** Associate Editor

Agricultural Research

Cover: Found in both rural and suburban areas of the southeastern United States, the imported red fire ant is a painful, costly nuisance.
Photo by Richard Nowitz (K-3637-1)

Page 13

Page 20



Page 24

Vol. 38, No. 7
July 1990

Editor: Lloyd E. McLaughlin
Associate Editor: Regina A. Wiggen
Art Director: William Johnson
Photo Editors: Anita Daniels, John Kucharski

Agricultural Research is published monthly by the Agricultural Research Service, U.S. Department of Agriculture, Washington, DC 20250. The Secretary of Agriculture has determined that publication of this periodical is necessary in the transaction of public business required by law of the Department.

Information in this magazine is public property and may be reprinted without permission. Photos (except those copyrighted) are available to mass media in color or black and white. Please order by photo number and date of magazine issue.

Subscription requests should be placed with the Superintendent of Documents, Government Printing Office, Washington, DC 20402. Please see back cover for order form.

Address magazine inquiries or comments to: The Editor, Information Staff, Room 316, Bldg. 005, Beltsville Agricultural Research Center-West, Beltsville, MD 20705. Telephone: (301) 344-3280. When writing to request address changes or deletions, please include a recent address label.

Clayton Yeutter, Secretary
U.S. Department of Agriculture

Charles E. Hess, Assistant Secretary
Science and Education

R.D. Plowman, Administrator
Agricultural Research Service

Robert W. Norton, Director
Information Staff

Worker fire ants attempt to groom and feed a surrogate rubber queen (left) scented with a fire ant queen pheromone. The strength of their response is compared to that of workers caring for a real queen at right. (K-3471-9)

Turning Up the Heat on Fire Ants

These fascinating little devils ruin parks and farmland, and they've even been known to wreck roads.

They are agricultural pests. They are medical pests. But transportation pests?

Strange, but true. Red imported fire ants, infamous for chewing up crops and leaving blister-like pustules on people and animals, have revealed yet one more odious habit: destroying roads.

They're not picky; they'll destroy either concrete or asphalt. But they have different methods for each.

On the Navy's Camp LeJeune in North Carolina, fire ants carried out bits of soil from under asphalt roads, forming intricate tunnels. They crawled underneath to keep warm in late fall and early spring. Then, when traffic or a heavy truck came along: instant pothole. The ants caused 160 potholes, each costing North Carolina's Department of Transportation $200 to fix.

On new concrete sections of Interstate 75 in Tampa, Florida, the ants had a different destructive approach: After entering naturally formed tunnels underneath the silicone sealant in joints between highway sections, they burrowed upwards. "Every so often, they'd feel the urge to come up," says William A. Banks, the entomologist who helped officials cope with the road destroyers.

Banks counted 226 holes in 3,085 yards of sealant. Repair costs range $132 to 301 per highway mile, and fire ant control is about $90 per mile each year.

In both cases, Banks, of ARS' Insects Affecting Man and Animals

Laboratory in Gainesville, Florida, recommended that officials kill the ants with a bait which contains the insecticide hydramethylnon.

Hydramethylnon, the insect growth regulator fenoxycarb, and the exotoxin abamectin (from the soil fungus *Streptomyces avermitilis),* were all discovered to be effective against fire ants by Banks and his colleagues in the late 1970's and early 1980's. Attractive baits that are widely used for fire ant control were developed as a result of their studies.

But one problem, he says, is that they are registered by the Environmental Protection Agency only for use in urban or industrial areas, not for agriculture. The manufacturers, with support from ARS and other scientists, have filed petitions to have fenoxycarb extended to agricultural land.

Farmers in the 11 states that fire ants currently infest may or may not have a problem with them. But crops in infested fields can suffer serious damage. "I know a farmer south of Gainesville who lost $25,000 or $30,000 worth of eggplants in one season," Banks says.

Fire ants, accidentally imported on cargo ships from Brazil or Argentina, are serious picnic wreckers, ferociously stinging animals and people. The hapless softball player who disturbs a fire ant mound stands to acquire numerous stings—up to 5 or 6 per square inch in a serious attack. Each sting may leave a painful blister called a pustule.

APHIS/USDA

In a typical infestation by large single-queen colonies, there may be 40 or more mounds per acre.

Nasty Little Wonders

If these insects weren't such a pain—literally—it would be easier to be fascinated by their social structure and behavior.

After a virgin queen mates in midair, she descends to the ground. No longer needing wings, she now chews or breaks them off. The remaining wing muscles are used as a nutritional source for her in establishing the new colony and in egg production.

The queen ant immediately searches for a place to hide herself—under a stone or a piece of paper, or in a clump of grass—"anywhere that she won't be obvious to birds or others that might feed on her," Banks says.

Once hidden, she digs a small burrow in the soil. Within 24-36 hours, she lays 50-60 eggs—some of them are fertile and will become ants; others, called trophic eggs, will serve as a sort of egg salad for the newly hatched larvae.

After hatching 5 to 10 days later, the white, legless larvae molt four times. They feed on the trophic eggs and on body fluids regurgitated by their queen. Finally, they enter a pupal, or dormant, stage. The first pupae to emerge are minims, very tiny workers who begin to enlarge the nest and bring in bits of food.

Meanwhile, the queen has laid more eggs, which the minims tend.

Within 25-30 days after the queen mates and begins the new nest, a small worker force is in operation. Thus begins a fire ant colony, unseen underground, that in a year's time may have 75,000 to 100,000 workers. Before dying out in 5-7 years, the colony may reach 200,000-300,000 ants. In a typical infestation by large single-queen colonies, there may be 40 or more conical soil mounds on 1 acre.

Minims initially remain close to the queen and developing young, feeding and tending them. With the hatching of the second group of eggs laid by the new queen, the minims begin to give way to slightly larger workers called minors. As the colony continues to grow and mature, still larger workers—medias and majors—appear. After about 6 months, the colony contains a wide range of worker sizes.

Worker ants care for and feed the queen and developing larvae, enlarge

APHIS/USDA

In suburban areas such as this cemetery, fire ant mounds are a menace to visitors and interfere with property maintenance.

and maintain the mound, search for and gather food, and defend the colony against intruders. The workers divide these tasks according to their age.

The youngest workers are the nurses, who move the immobile young around, feed them, lick them, and spray them and the nest area with a fine, anti-microbial mist of venom from the poison sac—all to keep them microbe-free.

As the workers age, they move away from the queen and the young and become reserves. They transfer food from the foragers to the nurses, keep the tunnels cleared of soil, and, when necessary, go out to do battle.

The oldest group of ants is the foragers. These are the workers who locate and bring in the food for the colony. These ants leave the nest through tunnels that radiate from the mound and have exit holes to the surface at various intervals. The foragers exit from these holes and search randomly for food.

ARS studies have shown that a forager who finds food returns to the hole, periodically touching its abdomen to the ground to leave a trail of chemical scents called pheromones. When the forager reaches the tunnel entrance, it emits a stronger dose of the chemical, the recruitment pheromone, which tells other ants "Hey, there's food out there."

They're pretty opportunistic. If it's edible, they take it.

After being recruited, the ants go marching one-by-one, following the pheromone trail, to find and bring back the hoard. If it's a large chunk, they break it up with their jaw-like mandibles and carry it back in pieces.

"They are very good at breaking up stuff that seems extremely hard. They can even burrow into bone," Banks says.

Only one stage of ant—the fourth larval stage—eats solid food. All the others thrive on liquids, sucking the juice out of food particles and tossing the remaining solid in the "kitchen midden," which is basically a garbage heap.

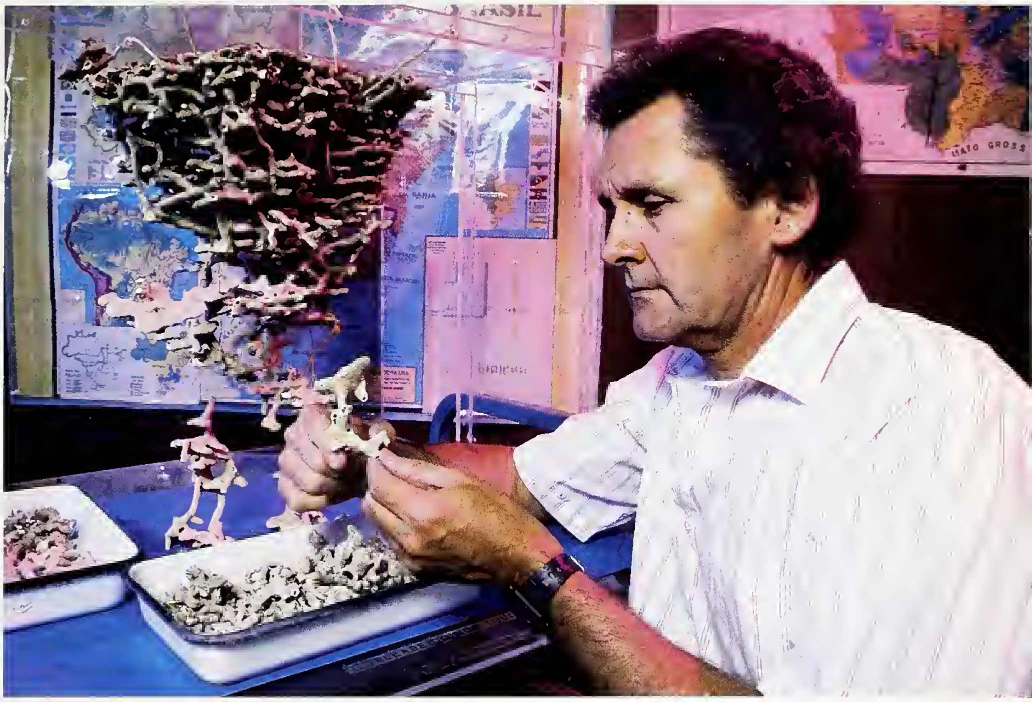
Banks says that fire ants eat mostly other insects, "but will feed on almost anything, from small animals and plant tissue to discarded human food. They're pretty opportunistic. If it's edible, they take it."

Citrus Villains

Their omnivorousness makes them frustrating in, for example, citrus groves. Fire ants love the sap that bleeds from the trees. When trees stop bleeding naturally, the ants chew away tree bark so more sap will flow. They can easily remove enough of a young tree's bark to kill it. Fire ants feed on branch tips, blossoms, and young fruit and are especially fond of navel oranges.

Even for mature citrus trees, the fire ants can spell trouble. They actually protect and tend aphids, mealybugs, and scales because these insects secrete a fire ant favorite—

ROB FLYNN



Entomologist David Williams assembles a plaster cast of an imported fire ant nest, revealing its channels, chambers, and intricate pathways. (K-2739-17)

honeydew. "Fire ants will build soil cages around these insects to protect them from their enemies and move them from place to place to keep them safe and well fed," Banks says.

Fire ants also damage soybeans, eggplant, corn, okra, strawberries, sorghum, potatoes, and pecans by feeding directly on the plants or by protecting other insects that damage the crops.

They interfere with farm operations. For example, when a soybean grower raises the combine cutter bar to avoid hitting mounds, soybeans go unharvested. A lowered bar gets the beans but also cuts through mounds, damaging the bar and contaminating the beans with soil and gravel. In this way, market price of the beans is reduced.

Nurseries that grow and ship ornamental plants have fire ant problems, too. The fear of receiving ants in trees, shrubs, and sod imported from infested states has prompted increased inspections and the threat of embargoes by western states such as Arizona and California. Ants found on a single plant may cause a whole truckload to be rejected.

All of this means higher production costs and smaller profits—hundreds of thousands of dollars—for growers in the 250-plus million acres currently infested by the ants.

A Spreading Nuisance

The red imported fire ant can infest and survive in roughly the southernmost third of the United States. Some officials have expressed concern, however, that the range could expand because of cross-breeding between red and black fire ants.

"The hybrid may be better able to survive in the border states such as Tennessee, Kentucky, North Carolina, Oklahoma, and others. Whether they will actually invade these areas remains to be seen," says ARS' Richard S. Patterson.

Scientists had thought the red and black fire ants were two different species. Because their hybrids produce viable offspring, the scientists now question if they are simply two races of the same species that vary in color. ARS will study this possibility, Patterson says.

Queens Galore

A comparatively recent discovery in the fire ant saga helps explain the insects' reproductive ruthlessness: their habit of throne-sharing.

Multiple queen colonies, first discovered in Mississippi about 16 years ago, are now known to occur in 8 of the 11 infested states. Each may have 10 to 100 or more queens, each reproducing. Multiple queens can mean up to 10 times more mounds per acre, studies show.

With single-queen colonies, any newly mated queen that lands in an area already infested is likely to be attacked and killed by worker ants.

"The biggest destroyer of fertile fire ant queens is other fire ants," Patterson says. He calls the single queen colony "a fortress unto itself."

But in polygyne colonies, instead of killing other ants, workers adopt them into their nest.

A worker fire ant (top) caring for an *Orasema* wasp pupa as it would for its own pupa in a fire ant colony. Micrograph courtesy of John Heraty, Texas A&M University. (90BW0330)

"If a newly mated queen comes down from the heavens," he reports, "instead of being killed, she is accepted by workers into a nest, where she sets up shop."

Studies by chemist Robert K. Vander Meer show that the ability of worker ants to discriminate between nestmates and fire ants from other colonies has been lost in polygyne populations, so that foreign queens are now accepted rather than being killed. Thus, fire ant populations of many acres act as if they were a single colony.

The Gainesville scientists are searching for more and better control methods. First they are developing more attractive baits.

The formulations now used for fenoxycarb and hydramethylnon baits are made of corn grits. They also contain soybean oil, which attracts many types of insects—including other species of ants that are, in many cases, "fire ants' greatest enemy," according to Patterson.

So not only are insects eliminated that would have naturally helped to control fire ants, but also some of the bait is removed that is meant for the fire ants.

Colony wreckers sneak into the fire ant nests and actually acquire the colony odor so that they will not be attacked and thrown out.

"Our goal is to develop baits that are more specific to fire ants," Patterson says.

Entomologist David F. Williams, for example, found that house fly and eye gnat pupae attract fire ants, which carry the pupae back to the colony as food. The scientists are investigating practical ways to use the pupae in baits.

In other work, chemist Robert K. Vander Meer has identified the recruitment pheromone. He hopes to combine this chemical and/or other pheromones produced by the ants with insecticides or insect growth regulators in baits that will attract only fire ants.

In addition to trying to expand the label uses for fenoxycarb, the scientists screen new chemicals that private companies send to them.

A Wasp That Busts Ants

Scientists working in biological control at the lab are looking closely at a parasitic wasp called *Orasema* and a parasitic ant, *Solenopsis (Labauchena)* species. Entomologists Daniel P. Wojcik and Donald P. Jouvenaz, in work with Vander Meer, found that these colony wreckers sneak into the fire ant nests and actually acquire the colony odor so that they will not be attacked and thrown out.

Fire ants integrate the invaders into the colony, deluded by the familiar odor. The *Labauchena* queen finds the fire ant queen and becomes yoked onto her neck, stealing food as workers bring it to her. The starved and weakened fire ant queen produces fewer workers.

BARRY FITZGERALD

Fire ants respond to various attractants in the prongs of this Y-tube being readied by biological technician Terry Krueger. (K-3474-3)

These organisms have never survived long in the lab after they were brought from Argentina or Brazil, but Juan Briano, who has reared them successfully in the ARS Hurlingham Laboratory in Argentina, will come north to tend them in Gainesville.

Making Ants Sick

Like all other animals, fire ants are subject to infectious diseases. In their native South America, they are attacked by viruses, protozoa, fungi, and nematodes. But when fire ants emigrated to the United States, their natural enemies were left behind.

A major goal of the Gainesville laboratory's biological control research is to introduce and establish in the United States a complex of fire ant diseases and insect enemies that could help to maintain a more natural balance in fire ant populations.

Some microorganisms attack a wide variety of insects and can be used for temporary, local pest control in the same manner as chemical toxicants. These microbial insecticides do not work with fire ants, however, because of a multitude of defenses. Chief among these is a filtration system in the throat of the worker ant that removes even bacteria. The result is that the queen—the reproductive center of the colony—is fed only highly filtered, microbe-free food and thus remains disease-free.

A second natural protective mechanism for both the queen and developing ant larvae: the antimicrobial aerosol emitted from the stinger of the worker that is periodically sprayed onto the brood, queen, and surrounding nest area.

Yet another defensive mechanism used by the fire ants is the tendency to move the nest to escape potentially damaging organisms. For example, when treated with such nonspecific organisms as nematodes, the fire ants will simply move the entire colony out of the treated nest and establish a new one nearby.

The establishment of a complex of natural enemies of fire ants in the United States may be effective enough to greatly reduce, if not eliminate, the dependence on chemical control.

Finally, Williams and entomologist Sanford Porter are investigating the reasons that fire ant populations may be extremely large in one field, yet very sparse or nonexistent in a seemingly identical field nearby. They hope to discover what natural factors prevent fire ants from thriving in such areas.

ARS hopes its multi pronged approach—chemical, biological, and biochemical—can help towards getting fire ants off our citrus trees, out of our back yards, and—yes—out from under our highways.—By **Jessica Morrison Silva,** ARS.

Scientists mentioned in this story may be contacted at the USDA-ARS Insects Affecting Man and Animals Laboratory, P.O. Box 14565, Gainesville, FL 32604 (904) 374-5900. ◆

Real-World Research: It's Pigs and Chickens

On a typical bright summer morning, Joan K. Lunney suits up in overalls and rubber boots. She's on her way to the pig barns.

Lunney isn't a farmer and the barns aren't ordinary production facilities, but what she is doing there could give farmers a chance to protect their herds from a costly swine disease—trichinosis.

Entering the barn where the pigs are kept three to a pen with plenty of room to move around, Lunney and two animal caretakers pick up the pigs one at a time and place them on their backs on a table.

An area on the neck is cleaned with alcohol and Lunney draws a small amount of blood with a syringe. The group then turns the pig right side up and returns it to the pen unruffled. Then they disinfect their boots in a germicidal bath and leave.

As an animal scientist, Lunney looks for immunity to trichina parasites among the genes of the pigs. She says that if immune cells play a role, then perhaps these cells could be used to rid the pigs of the parasite.

The blood collected today is from animals free of trichinosis. It will be processed and used as feeder cells for tissue cultures of T-cells—a type of white blood cell previously cultured from pigs infected with trichinosis. T-cells are specialized white blood cells that either attack and kill foreign invaders themselves or direct other protective cells that will kill these intruders.

"We are using the T-cells from infected pigs to see how they react to various stages of the trichina worm. We need to find out which ones react strongly.

The advantage of using cultures; they can give researchers a lot of answers in a shorter time without harming the pigs.

KEITH WELLER

Using a cannula, microbiologist Mark Jenkins (right) and assistant Marc Castle inoculate young chicks with a vaccine made from attenuated coccidial parasites. (K-3629-15)

Like many ARS scientists, Lunney reduces the number of animals used in research programs whenever possible. "In one year's time using cultures, we would need hundreds fewer pigs for research and could still obtain statistically and scientifically valid answers to such questions as, which of our pigs react very strongly to reinfection or infection with trichinosis? Which ones could be protected the most by a vaccine made with ground-up trichina worms or genetically engineered vaccines?"

Working with specially bred pigs, Lunney has shown for the first time that the worm that causes trichinosis can be evicted from the muscles of certain pigs.

Trichinosis infects several animal species, including humans. In humans, it is transmitted by eating infected meat that has not been properly cooked. In mild cases, the flu-like symptoms may go unnoticed. Only about 20 cases are reported each year in the United States.

Lunney is undertaking research with three strains of miniature pigs that were developed originally at the National Institutes of Health as genetic models for human organ transplants. Fully grown, these pigs range from 200 to 250 pounds compared with full-sized breeds that weigh in at 500 to 800 pounds or more. At NIH, some of the genes for mini-pigs were mapped—a plus for Lunney's research.

"We infected all three strains of pigs with trichinosis," she says. "When the trichina cysts were established in the muscles, we inoculated the pigs a second time. One of the strains of pigs reacted by destroying almost all the cysts in their muscles while the other two groups did not react."

"This is the reason we work with live animals. There is no way that present technology could have

predicted the destruction of the cysts using either computers or tissue cultures," says Lunney.

"We don't know exactly what caused the reaction, but we are pretty sure that immune cells played an important role in it. We hope our studies of the process will lead to a means of 'flushing' the trichina parasites from a pig's tissues before going to market."

That could open to U.S. farmers foreign markets that are now closed to fresh pork products. One result could be a one-third increase in export sales—added income for pork producers.

Lunney says the research with pigs could lead to a method to control other parasites that form cysts in muscles. For example, toxoplasmosis, a disease of pigs and other animals that is carried by cats, causes birth defects in humans and death in immune deficient patients. Once these parasites encyst, it is almost impossible to kill them with drugs.

NORITA CHANEY

What looks a bit like an animal's head is actually the tail of a *Trichinella spiralis* larva, magnified 1,600 times. It can infect the muscles of swine and a number of other animals as well as humans. SEM courtesy of J.R. Lichtenfels and Norita Chaney. (PN-7170)

Across and down the road from Lunney's laboratory at the Beltsville Agricultural Research Center works microbiologist Mark C. Jenkins. This morning, he and postdoctoral fellow Marc Castle are preparing to go to the chicken houses.

Jenkins, like Lunney, is a scientist who works on defenses against animal diseases. He is trying to find a vaccine against coccidiosis, a disease that robs American chicken producers of over $300 million annually in

Research with animals: What are the guidelines?

Recently, a growing public debate has focused on the use of animals in all kinds of research. As a result, a great deal of misinformation and confusion has been created over the need for such research and the prior view given to the use of animals.

In the Agricultural Research Service, all proposed research projects must be reviewed for quality and scientific merit by scientific experts.

If live animals are to be used in a project, an intensive Protocol Review must be followed. That requires the investigating scientist to develop a detailed description of all procedures that are to be used.

These Protocol Reviews are scrutinized by an Animal Care Committee at the ARS location where the research is to be conducted. This committee, which must have a minimum of 5 members, consists of other scientists, veterinarians, and nonscientist members from the local community.

The committee is required to inspect facilities where the animals will be housed and ensure that training on humane care of animals and regulatory requirements is available.

Likewise, the investigating scientist must assure the committee that the proposed experiments are not unnecessarily duplicative and that alternatives to using live animals were considered.

The investigator must show that the species of animal and number used are appropriate; that procedures avoid or minimize discomfort, distress, and pain; that any required surgery will be performed under proper surgical conditions and that veterinary medical care will be provided as necessary; and that the animals' living conditions are appropriate to that species.

Approval is valid for up to 1 year. New approval must be sought for any new procedures or to extend approval for procedures that take longer than 1 year.

All ARS facilities must adhere to Federal laws and ARS directives concerning animal care and use. Directives are regulations that apply to ARS personnel. Federal laws include the Animal Welfare Act and Endangered Species Act.

Guidelines that specify how animal subjects should be cared for include: the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and the *Guide for the Care and Use of Animals in Agricultural Research and Teaching.*

lost weight gain and medication costs. These hidden costs are actually paid by the consumer.

Jenkins also relies on live birds for his coccidiosis research, but first he screens just about all experimental vaccines in tissue culture. In preliminary screening tests, white blood cells from chickens that had coccidiosis and are now immune are mixed with the vaccine. If these immune white blood cells react to the vaccine preparation, then there is a reasonable chance that the vaccine will produce a similar immune response in the live bird. Only vaccines that excel in causing an immune response in this white cell model reaction are tested on chickens, says Jenkins.

"Our experience has also shown us that just because an experimental vaccine worked in our relatively small test flocks, it would not necessarily work in the field. That is why we must do extensive field studies before a vaccine can be released to the farmer."

Coccidiosis is insidious: Most infected birds look healthy, but the parasite prevents them from absorbing nutrients from the gut. "This causes a sort of slow starvation," says Jenkins.

Lately, Jenkins has been testing a coccidial parasite that is treated so that it can't reproduce, although it is still alive and able to penetrate a chicken's intestines.

The theory is that the chickens will react to the weakened coccidial infection with antibodies and immune cells, he says. It is hoped that when the chickens are exposed to the untreated parasite, their immune cells will be activated and the infection will be fought off.

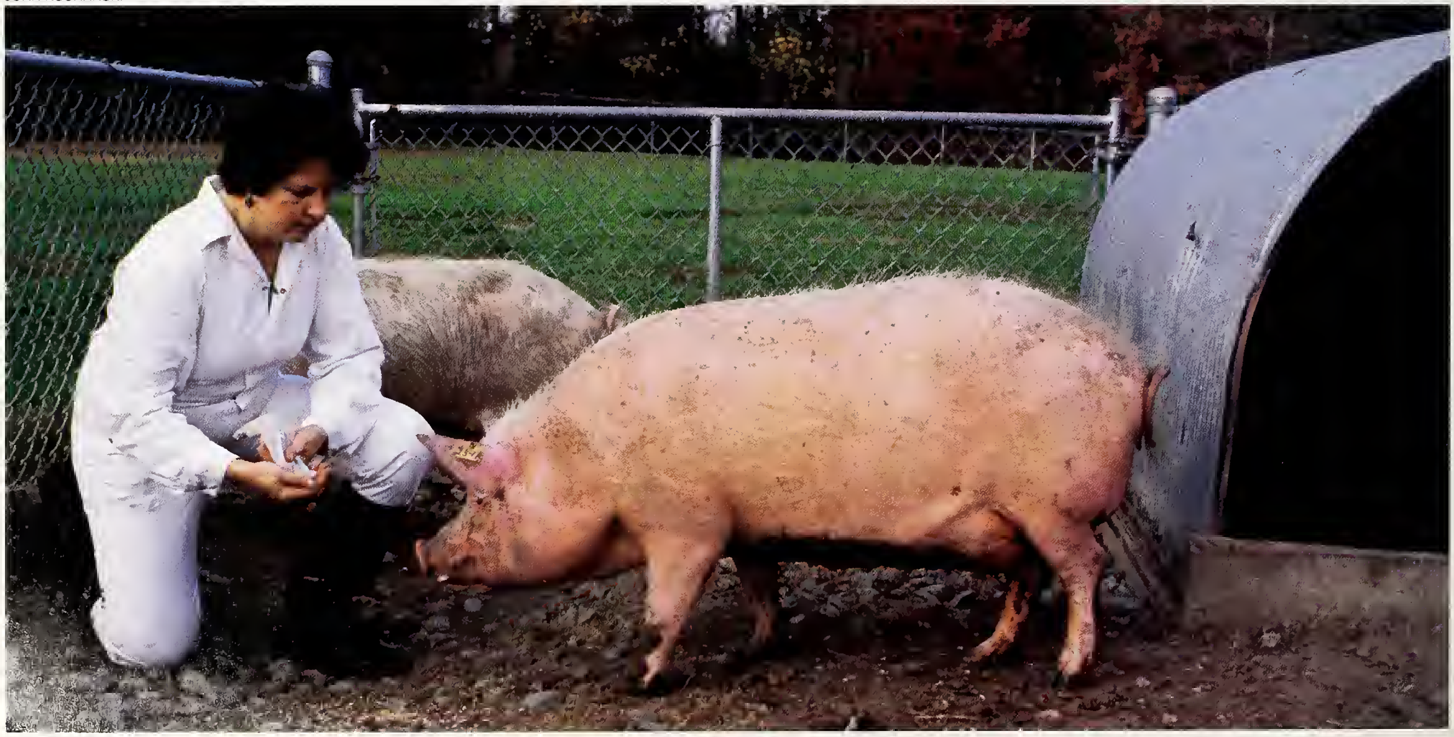
In one test, that is exactly what happened. Chickens that received the treated parasite were completely protected from infection while birds in a control group became infected.

But Jenkins cautions, "It is too early to celebrate or make predictions for a vaccine from these results. At the very least though, it tells us which form of the parasite is responsible for immunity."

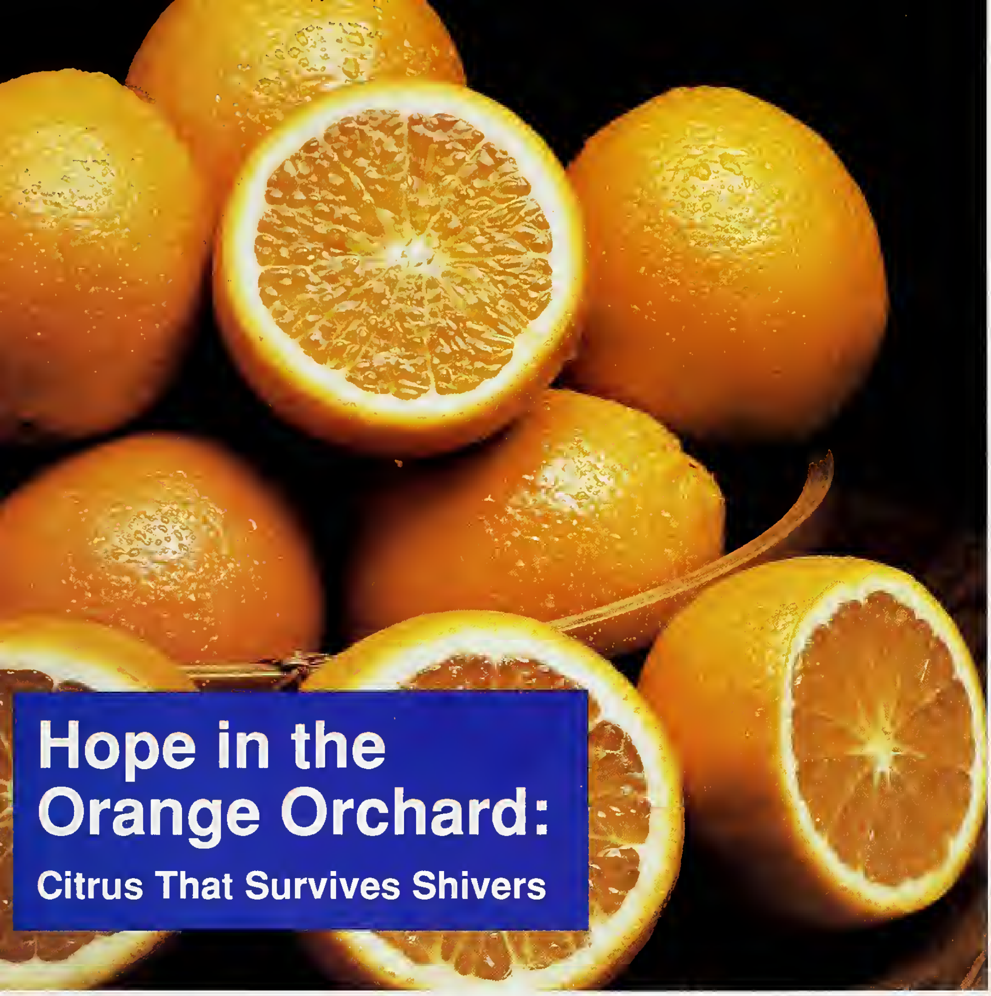
These two very different research projects both require healthy, well-cared-for animals or birds. This means good veterinary care, appropriate space for the animals or birds with proper lighting, humidity, temperature, ventilation, sanitation, and food supply.—By **Vince Mazzola,** ARS.

Joan K. Lunney is in the USDA-ARS Helminthic Diseases Laboratory (301) 344-1768 and Mark C. Jenkins is in the ARS-USDA Protozoan Diseases Laboratory (301) 344-8054) Both are at Beltsville Agricultural Research Center-East, Beltsville, MD 20705. ◆

JOHN KUCHARSKI



Animal scientist Joan Lunney is conducting research that may lead to eliminating trichina nematodes in pigs. (K-3406-13)



Hope in the Orange Orchard:
Citrus That Survives Shivers

Ambersweet, a new cold hardy orange variety. (K-3644-12)

RANDY SMITH

To bite into a new orange called Ambersweet is to sink your teeth into a smooth, firm, tasty fruit. Tightly packed juice sacs erupt, spewing forth their sweet juice. Pure pleasure.

There's more that's special about this orange; it grows on a cold-hardy tree, the only hybrid orange tree of its kind.

Good things are generally worth waiting for, but Ambersweet must have been a labor of love. It took plant geneticist C. Jack Hearn 26 years to bring forth this cold hardy-orange hybrid. Florida orange producers, accustomed to anxiously watching thermometers all winter, have long dreamed of such a feat.

"The last freeze we had, at Christmas time, proved that Ambersweet is well worth the time spent in developing it," Hearn says.

At test plots near Orlando where Hearn grows Ambersweet cooperatively with several local growers, temperatures fell to 18°F on Christmas Eve. "Ambersweet trees protected with heat or irrigation didn't drop their foliage. And, though unprotected trees lost foliage, they didn't suffer twig damage," he says.

Twig damage is the real demon. Not only does it cause fruit loss in the year of damage, but it can also carry losses over into the next year.

It can take 12 years or more to field-test it, produce fruit, and evaluate the results.

Hearn, located at the ARS Horticultural Research Laboratory in Orlando, says that most of Florida's fruit crop suffered damage in this freeze. If the groves had been populated with more cold-hardy citrus varieties, the damage would have been less severe.

Florida estimates its citrus is a $3.5 billion crop. Considering this vast economic magnitude, you may wonder why there aren't more answers to problems arising from four major freezes in the last 6 years? Why is Ambersweet the only cold-hardy variety?

"Because of the nature of breeding research," explains Howard J. Brooks, ARS national program leader for horticulture. "Growing citrus is not like growing row crops," he continues. "In 1 year, you can get three generations of tomatoes. But you don't just plant citrus and then harvest fruit the next year. Once you've developed a citrus selection, it can take 12 years or more to field-test it, produce fruit, and evaluate the results."

Hearn and Herbert C. Barrett, also a plant geneticist at Orlando, stepped up their efforts to breed cold hardiness into citrus in 1972.

Barrett has gained a wealth of knowledge from breeding tree fruits for 43 years. Twenty of those were spent working with citrus.

"Tree fruit breeding is different from any other kind of scientific research," Barrett declares. "In some respects, it's more complex than conventional projects, like putting a rocket in space."

Barrett defines a conventional research project as one from which a clearly defined objective can be stated and results can be shown in about a year. The whole process usually takes no longer than 3 years.

In tree fruit breeding, he says, you just make crosses and take what the genetics bear out. Then you make more crosses and play the waiting

RICHARD NOWITZ

game again, repeating until you get the results you're looking for.

According to Barrett, it's hard to maintain the validity of the research objective in tree fruit breeding because it takes anywhere from 15 to 25 years before results can be obtained. Because of all the variables that are being juggled, there's a higher degree of uncertainty.

"We began breeding for Ambersweet back in 1963 from a cross of sweet orange, mandarin, and grapefruit," says Hearn. It was not until 1989 that Ambersweet was released.

Granted, they were looking for a cold-hardy citrus, but several other objectives had to be considered.

"Of what use would a cold-hardy citrus tree be unless it produced edible fruit? Or, why have late-maturing fruit if the fruit freezes on a tree undamaged by the cold?" asks breeder Barrett.

Then there's the problem of searching among germplasm lines for sources of genetic material that carry desired traits such as cold hardiness, flavor, disease resistance, and size. If these characteristics can't be found in one source, then breeders must make their own through more genetic crosses. This means more time for plants to attain enough growth for desired traits to reveal themselves.

The lengthy research process began with crosses, then moved into actual fruit production, and ended with evaluation of those crosses—a step that can add 10 to 15 more years. Along the way, thousands of seedlings are discarded because they don't express the desired traits. Barrett estimates that only 1 seedling in over 1,000 will be released as a new, improved variety. This discard rate might be multiplied 10 times in breeding for a trait as complex as cold hardiness.

While a lot of conventional research is performed in the laboratory where conditions can be fairly well controlled, tree fruit breeding research is subject to many uncontrollable variables.

Plant geneticist H.C. Barrett examines seedlings of a cold hardy and disease-resistant hybrid created by crossing citrus with a citrus relative, *Poncirus*. The mature citrus tree in the background has survived all freezes in the past 15 years. It has been recently released as a breeding line, US119. (K-3644-6)

Barrett says one of the most important variables in citrus breeding is the environmental aspect.

"A severe freeze can kill trees planted in the field, wiping out 10 years or more of research effort," he says. Drought, water, and soil composition and condition all play an important role.

Citrus, says Orlando geneticist Donald J. Hutchison, is propagated by grafting an orange or grapefruit top, called a scion, onto an existing rootstock plant. It's the scion that determines what is produced. Since citrus shows much variation when grown from seed, seedlings must be vegetatively propagated.

Last year, Hutchison released a Chinese citrus rootstock that resists citrus blight, citrus tristeza virus, and phytophthora foot and root rot—diseases that plague Florida citrus .

"We tested this rootstock 14 years before releasing it commercially," says Hutchison. "We're just now beginning to see plantings of Sun Chu Sha."

RICHARD NOWITZ

At the ARS research station near Leesburg, Florida, plant geneticist Jack Hearn examines developing fruit on a 16-year-old Ambersweet tree. (K-3645-6)

He says most growers are still using rootstock developed from crosses made by Herbert J. Webber and Walter T. Swingle back in the early 1900's. USDA botanists Webber and Swingle helped establish the foundations of citrus research in Eustis, Florida, in 1892.

Once citrus hybrids have been field grown, breeders screen the seedlings for nematode and disease resistance. This takes about 3 to 5 years since some diseases don't express symptoms any earlier.

This, Hutchison says, eliminates about 95 percent of the seedlings.

From the seedling stage on, it's survival of the fittest. Researchers say the 1983, 1985, and 1989 freezes were helpful to breeders because they eliminated less-cold-hardy varieties that were being tested.

Along with Hutchison, Barrett and Hearn have scoured the world looking for germplasm with cold-hardiness and disease-resistant traits that might be compatible with citrus.

"We've brought back a microcitrus finger lime from New Guinea and a cold-tolerant desert lime from the outback region of Australia that sports thorny fingerlike spines. We're also intrigued by an Indian wood apple we found in Thailand that is capable of being grafted with our citrus," says Hutchison.

Crosses of these lines are growing at the research farm near Orlando. One cross that looks particularly promising is with *Poncirus trifoliata,* a native of Japan that is found in the United States. Very cold hardy, it grows as far north as Connecticut. Called trifoliate orange, it bears bitter, inedible fruit, but serves as a good rootstock for sweet orange.

Howard Brooks says ARS citrus breeders have made stalwart progress in developing more hardy citrus.

"We now have second and third generations of cold-hardy material that produce good quality fruit and resist a number of disease pests," he says. "It'll just be a matter of time now before the Orlando scientists are ready to release additional cold-hardy varieties."

In the meantime, the newest member of the Orlando breeding team is trying to bring biotechnology to citrus breeding.

"Now that the classical breeders have laid the essential foundation, molecular biology can work," says Randall P. Niedz. There are no transgenic citrus plants—yet. While tissue culture methods have cloned whole plants from single citrus cells, no foreign genes have ever been successfully transferred into citrus.

But the challenge excites Niedz. He and technician Delores Lomberk have already established cell lines capable of regenerating whole plants, critical to genetically engineering citrus. They plan to insert foreign DNA into these cells, hoping for regenerated plants that manifest changes from the DNA.

Brooks says Niedz' work bridges the gap between classical breeding and the research being done by pathologists and physiologists at the Orlando lab. While these researchers are discovering exciting new things about citrus, they still lack the means to transfer their results to live citrus plants.

For example, plant physiologist Michael G. Bausher has identified a protein in some *Poncirus* hybrids, developed by Barrett, that is not found in edible citrus. "We're anxious to see what effect this protein exerts on the plant's physiology, particularly cold hardiness, since it makes up about 20 percent of the plant's total protein," Bausher says.

So the heat is on Niedz to find a way to transfer genes into edible citrus. But first things first. Once Niedz' transgenic citrus plants express characteristics from foreign DNA, then he'll have the gene transfer mechanism.

Molecular biology, Niedz says, is a powerful tool that can realize the dreams of classical breeders.

"I'd venture to say that within the next few years, we'll have genetically engineered citrus."

"Imagine this," Niedz continues, "The Russians don't drink orange juice because it's too costly to import. A cold-hardy citrus would substantially increase production and lower import costs. So not only could the Soviets have a Big Mac for lunch, but they could also have imported OJ for breakfast!"—By **Doris Sanchez**, ARS.

The U.S. Horticultural Laboratory is located at 2120 Camden Road, Orlando, FL 32803 (407) 897-7300. ◆

DOUG WILSON

This, the second in a 3-part series, examines the diseases and pests that attack wheat and how agriculture's scientists are fighting back.

In the tumultuous world of international trade, with its swirling currents of rupees and rubles, dinars and dollars, one currency is universal: wheat.

As one of the world's most widely traded commodities, wheat claims a special position in the global agricultural economy—a fount of cash for national coffers as well as life-nourishing foods.

Happily for U.S. farmers, wheat is one currency that has been available to them in ever-increasing supplies. Although wheat was harvested from nearly 76 million acres in this country in 1949, compared with only 62.1 million in 1989, production last year totaled an impressive 2.035 billion bushels, nearly double the 1.1 billion of 40 years ago.

The difference has been a dramatic boost in yields per acre. But that victory has been hard-won. Diseases and pests have had to be defeated one by one in battles that have often pitted cutting-edge technology against age-old enemies.

The Agricultural Research Service's $21.3 million budget for

Although nonirrigated, the 8.5 million acres of hilly cropland in the Palouse area of Washington and Idaho boast remarkably abundant yields of wheat, barley, peas, and lentils. Indeed, no other area in the United States yields more wheat per acre under nonirrigated conditions. (K-1024-10)

WHEAT

Grappling With Ingrained Problems

wheat research in 1990 includes a war chest of about $3.8 million for finding ways to prevent or mitigate the ravages of insects and disease.

Fortunately for farmers and for mankind, science does not have to go it all alone. The same rich soil that supports the golden grain supports an unseen world of tiny organisms, a microenvironment vital for producing healthy crops.

Antibiotic Bacteria for Wheat

While the underground world is home to a host of fungi that can attack wheat roots, its residents also include beneficial bacteria capable of producing antibiotics to keep the bad fungi in check.

ARS plant pathologist R. James Cook hopes to help create a healthy balance of those organisms. Working at the Root Disease and Biological Control Research Unit at Pullman, Washington, Cook and colleagues David M. Weller and Linda S. Thomashow study strains of bacteria that team up to fight one of the most devastating fungal diseases of wheat—take-all.

The right bacterial mixtures can boost wheat yields 20 to 24 percent by stifling the bad fungi's spread.

A problem, though, has been in getting the right bacteria near the wheat roots where they do the most good. To track their natural movement in the soil, marker genes were inserted into a strain of one antibiotic-producing bacterium, *Pseudomonas fluorescens.*

The new genes have no effect other than to produce a bright blue compound when the bacteria are combined with certain chemicals in a lab dish. By putting the altered *P. fluorescens* in a test plot planted with wheat, the scientists were able to track its movement by periodically sampling soil and roots.

"We found that the bacteria didn't spread from row to row, but they did spread from plant to plant in the same row," says Weller.

Cook says Thomashow has cloned antibiotic-producing genes from the bacteria, and these genes have been expressed in unrelated bacteria, including strains of bacteria from roots of wheat. "Maybe someday, we'll be able to move those genes into wheat so the wheat can make its own antibiotic," he says.

Rust Resistance

Other fungi, the ones responsible for cereal rusts, are keeping scientists busy at the ARS Cereal Rust Laboratory at St. Paul, Minnesota.

Each year, the researchers travel thousands of miles, mostly in the Great Plains Wheat Belt, collecting infected leaf and stem samples and judging how well grain varieties resist rusts. They study rust fungi from these samples and others sent by researchers in the United States, Mexico, and Canada to try to predict what might happen next with these diseases.

"The vigilance of our scientists helps plant breeders get a head start on developing new resistant wheats," says Kurt J. Leonard, director of the St. Paul laboratory.

In the past, scientific teamwork against these diseases has paid off well. In 1916, wheat stem rust destroyed about 38 percent of the U.S. wheat crop. But during the past 35 years, wheat yield losses attributed to stem, leaf, and stripe rusts have ranged below 1, 6, and 2 percent of crop yields, respectively.

Still, the danger is not over. According to plant pathologist Alan P. Roelfs of the Cereal Rust Laboratory, epidemics could recur within a few years unless research continues to develop grain varieties with several genes to impart resistance.

The extent of the rust threat is also affected by changes in cropping practices and the number of acres

DAVID WELLER

Marker genes genetically engineered into *Pseudomonas fluorescens* allow scientists to study movement of these beneficial bacteria in the soil. Blue outline to the wheat root (right) indicates the presence of the altered bacteria.

DAVID WELLER

planted. For example, Roelfs says, widespread irrigation in the central Great Plains leaves more moisture in the soil when the plants are mature. This, coupled with the normal scattering of wheat kernels on the ground during harvest, has led to an increase in the number of volunteer plants that survive the summer. These serve as ideal hosts for leaf and stem rusts, carrying the infection over to later plantings of winter wheat.

By enlarging plantings of winter wheat in traditional spring wheat areas of the Northern Great Plains, growers may inadvertently be increasing the presence of leaf rust, Roelfs says.

After being protected through the winter by snow cover, rust from winter wheat plantings could thrive and reproduce rapidly in an unusually warm spring. The threat of leaf rust infestations may also be heightened by such cultural practices as minimum tillage, which enhances snow cover, he says.

The news regarding rust isn't all bad, though. A research team led by plant geneticist Norman D. Williams at the ARS Northern Crop Science Laboratory at Fargo, North Dakota, has turned up indications of stem rust resistance not recognized previously in known wheat varieties.

Plant pathologist James D. Miller contributed by developing a virulent rust strain from four races grown in the laboratory on an alternate host—barberry—under quarantine.

The new race is genetically similar to—but is much more virulent than—race 15TNMK, the predominant rust race in states ranging from Texas to Minnesota.

In tests, the wheat variety Waldron succumbed to the new rust strain even though it is a prized progenitor of new varieties, with at least four known rust-resistance genes and other desired qualities. But varieties Len, Coteau, and Stoa survived the virulent strain, implying that they have unrecognized resistance.

As with rust research, the study of septoria leaf blotch and tan spot, two other major fungal diseases of wheat, has resulted in new questions about cropping practices.

Septoria leaf blotch, caused by the fungi *Septoria tritici* and tan spot, caused by *Pyrenophora tritici-repentis,* are common in many parts of the world.

DAVID NANCE

As Russian wheat aphids feed, the wheat leaves tend to roll around them making them difficult to kill with conventional pesticides. (K-3656-1)

Grain losses from leaf blotch frequently range as high as 20 percent. Tan spot, found in the United States only in the last 20 years, reduced yields by at least 30 percent in stubble-mulched fields in South Dakota in 1977.

Francis J. Gough, an ARS plant pathologist at Stillwater, Oklahoma, found that no-till wheat grown each year amid the leaf blotch- infested straw of the previous year's crop at Stillwater and Altus, Oklahoma, at first had more septoria leaf blotch lesions than wheat planted where soil had been plowed, disked or subsurface-tilled with a V-shaped blade.

But that trend inexplicably started reversing in May, leaving the no-till wheat no worse off by harvest time. By contrast, tan spot was more severe in no-till wheat because of the presence of infected crop residue on the soil.

In other field tests, Gough found that wheat leaves harbor beneficial bacteria that seem to check the leaf blotch and tan spot fungi.

Perhaps someday genetically engineered strains of beneficial bacteria such as *Pseudomonas fluorescens* will more effectively counter both tan spot and leaf blotch.

"We may even breed wheat that will resist fungi and at the same time support large populations of beneficial bacteria to control diseases," Gough says.

Alleviating Aphids

Not all threats to wheat come from disease; pests also play an important role in the fate of U.S. cereal crops. Currently holding center stage is the Russian wheat aphid, the newest threat to wheat, other small cereal grains, and even some forage grasses.

First spotted in Texas in 1986, the Russian wheat aphid has since cost farmers in 16 western states more than $200 million in damage and control.

Conventional insecticides don't work well here. After sucking juices from the base of plant leaves, the aphids lie protected by the developing leaves that tend to stay rolled around them.

"The most realistic way to combat Russian wheat aphids appears to be integrated pest management," says

Robert L. Burton, ARS technical coordinator for Russian wheat aphid research and director of the ARS Plant Science Research unit at Stillwater, Oklahoma.

Scientists at Stillwater have found Russian wheat aphid resistance in some barley, wheat, and wild plant species related to wheat, rye, triticale—a manmade cross of wheat and rye—and forage grasses.

Next question for researchers: Is the resistance genetic, biochemical, physical, or a combination?

Some of the parasites, predators, and pathogens that normally prey on the Russian wheat aphid in the USSR and in other countries have been located by scientists based at the ARS European Parasite Research Laboratory at Behoust, France.

From Behoust, these potential biocontrol agents go to the ARS Beneficial Insects Laboratory at Newark, Delaware, and to a Texas A&M University laboratory at College Station, Texas, for quarantine. Once through quarantine, they are distributed to researchers with ARS, as well as USDA's Animal and Plant Health Inspection Service, and other cooperating agencies.

So far, ARS scientists at Ithaca, New York, have found that the fungus, *Pandora neoaphidis,* is the most common pathogenic fungi living on aphids in North America and Eurasia. Preliminary tests suggest the Russian wheat aphid also is susceptible to a wide variety of fungi that attack other aphid species.

Cultivating Pest Control

Cultivation practices not only play a role in disease problems in wheat, but also can affect pest attacks, researchers are finding.

Greenbug, a small green aphid, is apt to fly right past some of its favorite crops if the soil hasn't been recently tilled. This is because the glare of short wavelengths of light reflected from unplowed soil and crop residues interferes with the migrating greenbugs' ability to find a succulent dark-green crop.

Being snubbed by greenbugs is a bonus for Great Plains grain farmers who alternate wheat and sorghum and idle the soil for about a year between crops with minimal tillage to control weeds.

Burton and coworkers studied the effects of farming practices on greenbugs for 7 years at sites ranging from Bushland, Texas, to North Platte, Nebraska.

They found that three practices consistently thwarted the greenbug: no-till farming, planting greenbug-resistant crop varieties, and waiting until after the peak migratory flights of the pest before planting sorghum.

"Where we used all three practices, greenbug numbers were always too few to warrant chemical controls, reducing the need for expensive insecticides that may threaten the environment," Burton says.

Virus Carriers

The line between pest problems and disease problems becomes somewhat blurred in the case of barley yellow dwarf virus (BYDV), another devastating aphidborne wheat disease agent. The virus takes its toll among all grain crops—not just wheat—with losses ranging from 5 to 90 percent.

"Aphids occur in great numbers, reproduce extremely fast, and transmit the virus so quickly that you'd have to spray crops almost daily to prevent its spread," notes Stewart M. Gray, a plant pathologist at the ARS Plant Protection unit at Ithaca, New York. Instead, Gray hopes to halt the

DAVID NANCE

To compare different wheat plants for their ability to host the bacterium *Pseudomonas fluorescens,* which fights septoria leaf blotch, plant pathologist Francis Gough collects wheat leaves that have been sprayed with the bacterium. (K-3658-1)

DOUG WILSON

Engineers Keith Saxton (left) and John Driessen note the reduced soil and stubble disruption from a cross slot drill compared to the USDA II No-Till drill (background). (K-2815-7)

virus by slowing its spread from infected plants to healthy ones.

"Before we can develop methods to control aphid transmission of the virus, we must first understand the relationship between the virus and aphid," he says. "It's not a simple system. There are different types of BYDV, and each one is spread by a different aphid species. We're trying to figure out why that aphid-virus specificity exists."

Gray says the answer may lie in the protein shell of the virus itself. Each type of BYDV has within its shell crucial amino acids that enable the aphid to recognize and transmit its particular brand of BYDV to a vulnerable crop plant.

The transmission cycle begins when an aphid feeds on an infected plant. The virus goes from the plant into the aphid's digestive system, from there into the insect's blood, and within about 24 hours winds up in the aphid's salivary glands. The aphid is then ready to pass the virus along as it feeds on another, perhaps uninfected plant.

Gray says that if the correct amino acid sequence is not present and recognized by virus receptors in the aphid, the pest cannot pass along that particular strain of BYDV.

Gray and fellow researchers at Ithaca have identified several monoclonal antibodies that can block transmission of certain types of the virus. The monoclonal antibodies do this by linking with the key amino acids so they cannot connect with the receptors in the aphid's body.

Next, the scientists hope to pinpoint precisely which amino acids have been recognized and tied up by the monoclonal antibodies—the amino acids that are normally responsible for transmission of that particular type of BYDV.

Despite the successes so far with monoclonal antibodies, "Control in

the field really is still a long way down the road," Gray says.

The Land Beneath the Crop

Even if science somehow conquers the pests and diseases that plague wheat, will yields still trend higher as they have in the last 4 decades? Or will erosion and loss of soil fertility end our record harvests?

Donald K. McCool, an agricultural engineer, is helping answer these questions. His work at the ARS Land Management and Water Conservation Research Unit at Pullman, Washington, focuses on better ways to predict soil losses.

Crop residues help curb erosion induced by rain and snow melt on thawing soil on the sloping farmland of eastern Washington and northern Idaho. According to McCool, new evidence shows that less crop residue can be left on fields if farmers also practice other plowing and planting techniques that keep soil in place.

One promising technique is using the cross-slot drill. This planting method was originally developed by researchers at Massey University in New Zealand and recently modified and tested by agricultural engineer Keith E. Saxton of the ARS Land Management and Water Conservation Research unit at Pullman, Washington.

The cross-slot drill can slice through residue-covered fields, depositing fertilizer and seed in a single pass.

The drill features a unique soil opener that keeps crop residues on the soil surface to help reduce erosion. In addition, yields in 1988 for some 250 test plots were 15 percent higher with this drill than with the double-disk yielder drill.

Saxton works closely with local growers to transfer the technology from researchers to industry to farmers. As a result of collaboration between scientists with ARS, Massey University, and a Seattle-based company, AgriSystems, farmers in the Palouse region of eastern Washington tested the drill on 1,100 acres of cropland last fall.

"Farmers respect data from researchers, but we want to see how it works on our own ground," says Dick Druffel, a Pullman farmer who planted 350 acres of his land with the cross-slot drill last fall.

The drill cut through residues well, he reports, and emergence of viable seedlings was also good.

Druffel's favorable impression of the drill will be passed on to other farmers. He says, "Everybody here shares their experiences about new, better methods with their neighbors."

DOUG WILSON

Focusing on better ways to predict soil losses, agricultural engineer Donald McCool inspects a test plot where stubble has been removed for studies on soil erosion. (K-2809-2)

Balancing Nitrogen Fertilizer

Wheat thrives on nitrogen—the right amount of nitrogen, that is. More than is needed can be bad news for farmers and the environment.

Excessive nitrogen boosts wheat protein levels, an undesirable condition for soft white winter wheat used for cakes, pastries, and oriental noodles. In addition, nitrogen that's not sopped up by the growing wheat plant can seep through the soil, possibly contaminating groundwater.

Researchers aren't sure exactly where the nitrogen is going, or when, orhow long its journey takes. To answer these questions, soil scientist Jeffrey L. Smith of the ARS Land Management and Water Conservation unit tracks nitrogen movement through the environment by adding a stable isotope, 15N, to fertilizer.

ROB FLYNN

ROB FLYNN

▲ **Underground wheat roots viewed from the minirhizotron's video monitor. (K-3636-19)**

◀ **Soil scientist James Box (foreground) inspects the tip of a video probe which contains the lights, lens, and imaging sensors to be inserted into the minirhizotron tube by technician Johnny Doster. (K-3636-11)**

It's a naturally occurring, non-radioactive form of nitrogen that serves as a marker.

Smith mixes the 15N into fertilizer, injects it onto test plots, and then samples both soil and plants to trace the nitrogen's path. By calculating the amount of isotope recovered, he can determine how much of the fertilizer and soil nitrogen the crop has used. The remaining fertilizer nitrogen can be followed through the soil environment.

Smith believes environmental conditions influence wheat protein levels much more than plant genes do. Yearly variations in rainfall patterns, for example, can affect nitrogen movement, and consequently, protein levels.

"The high protein levels in winter wheat may be related to heavy rains in late spring," says Smith. His 15N-marked-fertilizer studies indicate that leaching is significant in the spring. It can flush heavy applications of fertilizer nitrogen deep into the soil, where plant roots shoot it straight to the grain as the crop approaches maturity.

Once Smith completes his experiments, he'll be able to advise wheat growers on specific management practices, such as the amount and timing of fertilizer to maximize efficient use and minimize losses.

Wheat and Waterlogged Soils

Environmental problems of a different sort occupy the attention of

soil scientist James E. Box, Jr., at Watkinsville, Georgia. He's looking for a soft red winter wheat plant that will take to Georgia's waterlogged winter soils like rice in a paddy.

He's able to evaluate the ability of wheat plants to survive in watery settings by studying their roots with a minirhizotron. This instrument probes the underground world of roots with a small camera designed for medical use with scopes inserted into the human body.

Box tests each wheat variety in both waterlogged and nonwater-logged soil, counting the number of roots that come within camera range. Using this information, he has developed a formula for converting root count into root length per volume of soil, traditionally called root length density.

The mathematical conversion can be done in seconds and eliminates the laborious task of digging down several feet to remove a soil core, washing it to expose the roots, and then stretching out the roots and measuring them.

Box and University of Georgia colleagues Jerry Johnson and Bill Hargrove plan to screen about 800 varieties. "Once we find plants with the best ability to grow with little or no oxygen underground, Johnson will breed that characteristic into a wheat variety that grows well in the Southeast," he says.

The information on root turnover plus the root length density data produced by the minirhizotron are already serving another purpose. It's being incorporated into a computer model that will someday help farmers predict yields of soft red and soft white wheat types under a variety of conditions.

Already, a computer program known as PLANTEMP can predict how a wheat crop will respond to nature's ways. Now, an outgrowth of PLANTEMP may foretell the consequences of some unnatural environmental effects—like global warming—on wheat crops.

DAVID NANCE

Entomologist Robert Burton examines aphid cages to monitor the effects of various light wavelengths. Light reflecting from straw, soil, or mulch around a plant has an effect on aphid reproduction, longevity, migration, and host acceptance. (K-3658-13)

Designed by ARS scientists in Pendleton, Oregon, PLANTEMP predicts when wheat shoots, roots, and leaves will appear, based on average high and low daily temperatures. The new model, known as "Modular Winter Wheat," will also predict when spikelets—which contain the wheat grains—should appear, and the size of each plant part, according to soil scientist Ronald W. Rickman.

Determining plant part size requires taking into account photosynthesis rates as well as temperature measurements. These rates depend on factors such as the availability of light, water, and carbon dioxide.

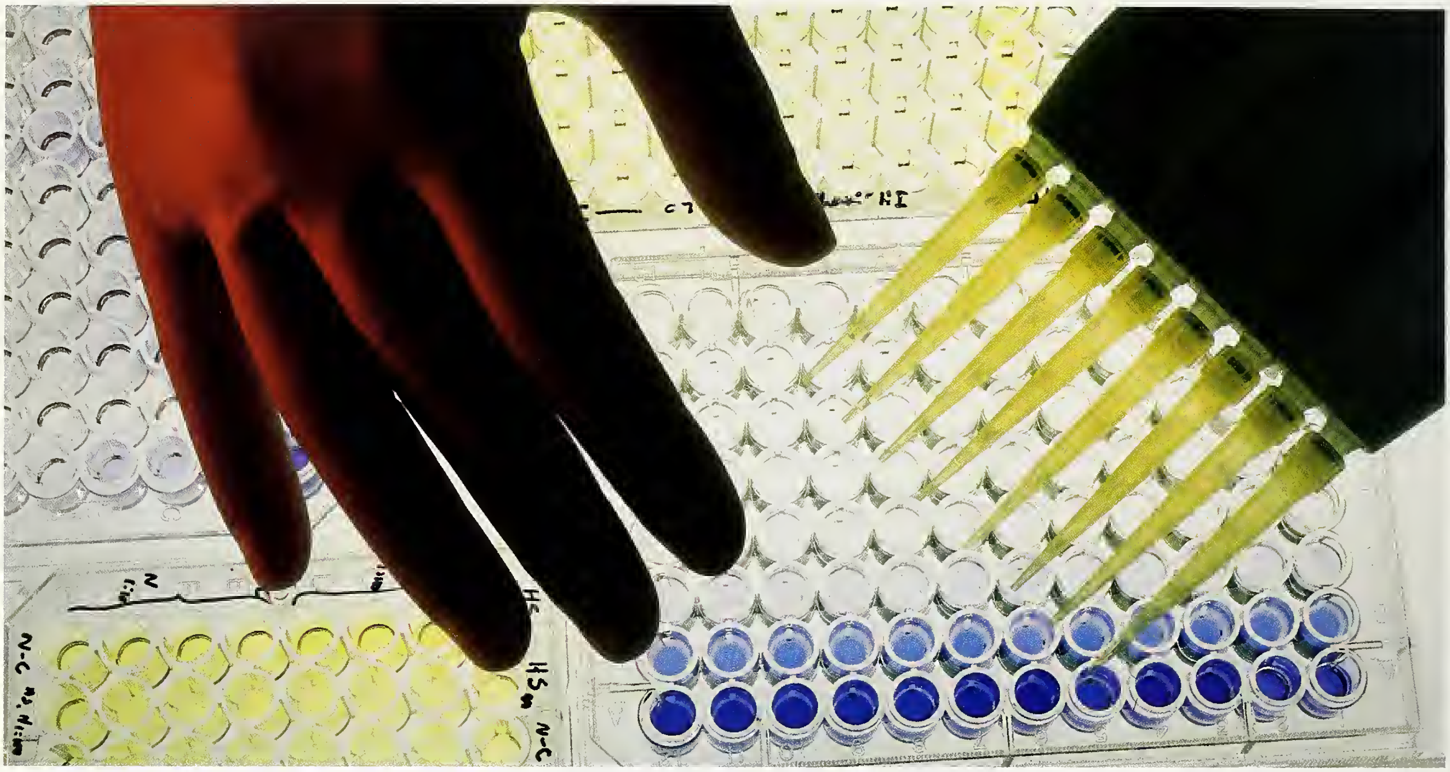
"Knowing both the development and growth of wheat parts can help farmers decide when to apply spring fertilizer to get the most from their crop," says Rickman, who formulated both PLANTEMP and the revised version with plant physiologist Elizabeth L. Klepper. Rickman and Klepper work at the ARS Columbia Plateau Conservation Research Center at Pendleton.

Besides optimizing fertilizer use, the program could also simulate what may happen to wheat if global temperatures creep upwards—a scenario many scientists fear will occur as atmospheric carbon dioxide levels increase.

The model is still being evaluated by researchers in Mississippi, Georgia, and Colorado with data generated at those locales. Rickman expects field tests to be under way next year, and he hopes the program will be available to researchers and farmers in 2 years.—ARS writers **Julie Corliss, Ben Hardin, Don Comis,** and **Sandy Miller Hays** contributed to this article.

Next month: Decoding the mysteries of genes and proteins and protecting the wheat harvest. ◆

RICHARD NOWITZ



Genetically engineered *E. coli* bacteria activate an enzyme that produces a blue color in this SOS Chromotest. The color is proportional to the amount of DNA repair taking place. The test can be used to assess how diet influences a person's risk of developing some forms of cancer. (K-3543-3)

Defining the Link Between Diet and Cancer Risk

Risk assesment relies on educated guess, especially in the case of cancer where the disease may take a generation or more to express itself.

The more precise the data used, however, the more accurate the guess. In a few years, physicians and scientists may have a tool to better assess how diet influences a person's or a population's risk of developing some common cancers well before the disease arises. And the same tool may shed some light on whether or not the presence of pesticides or natural chemicals in groundwater increase our risk of cancer.

"We have suggested a biochemical basis for the link between high-fat diets and the chance of developing colon cancer."

Padmanabhan P. Nair

In an Agricultural Research Service laboratory at the Beltsville Human Nutrition Research Center in Maryland, biochemist Padmanabhan P. Nair and colleagues have adapted a French-developed test—which measures genetic damage in special bacteria—to indicate an increased potential for colon and possibly other types of cancer.

If the new technique continues to prove reliable in further studies, says Nair, "it should give physicians a more solid basis for advising individuals to modify their diet. And it should enable scientists to survey large populations and develop a national map showing relative risk rates in various geographic areas."

He led a group of researchers with ARS and the Johns Hopkins University in adapting the SOS Chromotest to study how dietary fat influences risk of colon cancer.

The test uses a genetically engineered *E. coli* bacterium to measure breakage, or mutations, of DNA. "This is a necessary step in the transition of cells from a normal to a malignant state," he says.

But how does one sort out the factors in a complex diet that could promote cancer? In the early 1980's, Nair directed a 5-year study of diet and cancer incidence comparing vegetarians, who ate less than 30 percent fat, with the general population living in Los Angeles.

The study showed that high-fat diets prompt intestinal microorganisms to produce more secondary bile acids, which are part of the fatty substance in stools that cause mutations. Also, the study found that the level of these acids is a good indicator of risk for colon cancer. Several other studies have corroborated these findings.

With this knowledge, he and assistants Samina Shami and Eduardo Sainz extract all the mutagens from human stools, apply the extract to the special bacteria, and measure the number of mutations. When 31 women in a controlled dietary study cut their fat intake in half—from 40 percent down to 20 percent of calories consumed—their stool extracts produced only half as many mutations, says Nair. Current studies of stool extracts from men are yielding similar results.

"We have suggested a biochemical basis for the link between high-fat diets and the chance of developing colon cancer," he says. "While mutations don't automatically lead to cancer, we believe they clearly increase the risk. This has been established in animals but is still under study with humans."

He predicts that testing stool sample extracts may also prove to be a good indicator of risk of breast and other cancers. That's because fatty mutagens in the colon can be reabsorbed and deposited in the breast and other organs having fatty tissue. And most cancers occur in cells similar to those that line the colon.

The rationale is that mutations, or breakage, of DNA can lead to malignancy. Before a normal cell can become a cancer cell, inactive cancer-promoting genes known as protooncogenes have to be cut and spliced into a new place on the DNA to become active oncogenes, he explains. "That's like rearranging the garbled letters of an anagram to spell a word that makes sense."

The more breaks in the DNA, the greater the chance the dormant protooncogenes will be rearranged into active oncogenes as the huge molecules are spliced back together.

In the SOS Chromotest, the genetically engineered *E. coli* bacteria signal DNA repair by activating an enzyme that generates a blue color. The strength of the color is proportional to the amount of enzyme produced, so the tester can measure breakage by using a color standard produced with a known mutagen.

Similar tests, such as the Ames test, distinguish chemicals that cause mutations (mutagens) from those that don't. But they don't indicate the amount of breakage, says Nair.

He hopes to train health-care faculty from universities and medical schools in how to achieve accurate results with the screening test. They, in turn, can teach their colleagues and students.

"We should be able to project how a change in diet will change risk," he says. "We've done it in the lab, but we need to test it nationally."

Others involved in the research include Joseph T. Judd and George P. Albaugh of the Agricultural Research Service, and Lois B. Jerabek, Vasantha Iyengar, Samina Shami, and Althaf Lohani of the Johns Hopkins University.

Diet is only one aspect of risk, he says. Other aspects are heredity, factors that activate foreign cancer-promoting genes present in human

RICHARD NOWITZ

ARS chemist Padmanabhan P. Nair (foreground) examines an SOS Chromotest plate at his Beltsville, Maryland, laboratory. (K-3542-13)

cells, and exposure to cigarette smoke and other environmental carcinogens.

Within the next year, Nair hopes to add other tests for assessing the total risk profile for cancer. "The adapted chromotest is only one quarter of the total picture," he says.

The SOS Chromotest was developed at the Pasteur Institute in France primarily to screen industrial compounds for mutagenicity, says Nair. So he introduced the test to environmental chemist Jack R. Plimmer at the Beltsville center, who was looking for a way to assess groundwater for cancer risk.

Nair's group is measuring mutagenicity of groundwater samples collected from selected locations, while Plimmer's group is analyzing the samples for total carbon—the backbone of all organic compounds.

Plimmer's lab will also analyze for pesticides and other carbon-containing pollutants. "This test correlates very positively with mutagenicity," he says.

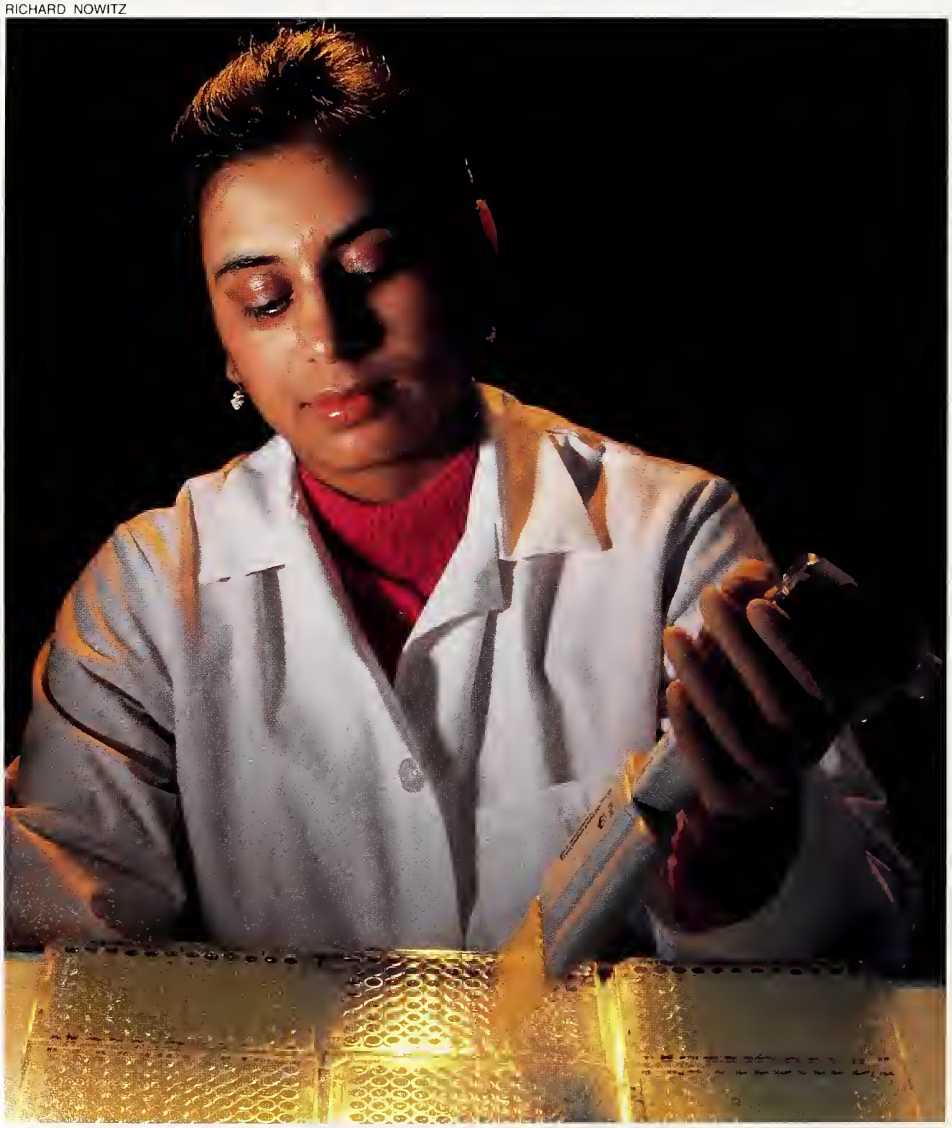
With $45.4 million in new appropriations to protect groundwater, USDA recently launched a 5-year plan to reduce the presence of pesticides and fertilizer. Doral Kemper, who oversees ARS' soil management research, says if groundwater were tested nationwide, then a national map of mutagenicity could be developed.

He says such testing could gauge the risk posed by pesticides against that posed by natural compounds, such as those released by decaying plants. According to ARS water analysts, Kemper says, pesticides account for less than 1 percent of all organic compounds in groundwater, lakes and streams. And Bruce Ames of the University of California, Berkeley, developer of the Ames test, concludes that natural compounds in water are about as likely to cause mutations as manmade compounds.

Kemper notes that filtration plants remove many of these organic compounds from city water.—By **Judy McBride,** ARS.

Padmanabhan P. Nair, is at the USDA-ARS Lipid Nutrition Laboratory, Beltsville Human Nutrition Research Center, Beltsville, MD 20705 (301) 344-2145. ◆

RICHARD NOWITZ



Research associate Samina Shami prepares SOS Chromotest plates, which use genetically engineered bacteria. (K-3541-11)

Tanks for Saving Water

Simple, cheap, easy-to-make-at-home covers for livestock water supplies are cutting water losses at more than 100 locations in the United States.

"Throughout much of the West, the covers can prevent up to 4 feet of water from evaporating each summer from open-top, vertical-walled storage tanks. Even more water is saved in warmer climates," says Allen R. Dedrick, ARS agricultural engineer at the U.S. Water Conservation Laboratory, Phoenix, Arizona, who pioneered the research in the early 1970's.

The covers are made from rolls of 1/4-inch-thick by 4-foot-wide foam rubber that is light enough to float on water but heavy enough not to be blown off by normal winds. Cutting and bonding with contact adhesive, it takes two workers about 3 hours to construct a cover that's 30 feet in diameter.

They cut 1/2-inch-diameter holes every 3 to 4 feet along each sheet to allow rainfall to drain into the tank. In particularly windy areas, the edges can be weighted to keep the wind from flipping the covers off the tanks and wires can be stretched across the top.

According to a recent followup survey, the covers have withstood the ravages of weather up to 10 to 15 years.

"Wildlife managers are enthusiastic about the cover because they serve as watering rafts for birds. I've seen hundreds of doves around the tanks and floating on the covers," says Dedrick.

He continues to fill requests for instructions to build the covers.—By **Dennis Senft,** ARS.

Allen R. Dedrick is in USDA-ARS Irrigation and Hydraulics Research, U.S. Water Conservation Laboratory, 4331 E. Broadway Road, Phoenix, AZ 85040 (602) 379-4356. ◆

Wilt-Not Maples

Norway maples—graceful, compact trees that thrive in urban areas—are one of the five most popular street and shade trees in the United States. Unfortunately, these trees are often damaged or killed by *Verticillium* wilt.

But Norway maples resistant to *Verticillium* wilt are now a distinct possibility, thanks to the work of ARS scientists Alden M. Townsend and Lawrence R. Schreiber.

Currently, there is no known control or treatment for the disease, which is caused by a microscopic fungus that generally lives in soil.

"All you can do right now is prune out infected parts, water and fertilize the tree, and hope for the best," explains Townsend, a plant geneticist with ARS' National Arboretum in Washington, D.C.

So Townsend and Schreiber started searching among the 13 common varieties of Norway maples for ones with natural resistance to the wilt.

They found two varieties, Jade Green and Parkway, that were not so much resistant as they were tolerant to the fungus.

Technically, being able to withstand the presence of the fungus is considered tolerance, while resistance would be if the organism were not able to infect the tree at all, Townsend explains.

"Jade Green and Parkway Norway maples were able to harbor the same level of infection by the fungus while showing significantly fewer signs of the disease when compared to the most susceptible varieties—Crimson King and Greenlace," Townsend says.

It was the wide variation in response to infection that Townsend found most encouraging.

"The amount of variation means there is definitely potential for improvement by selective breeding—potential to breed a Norway maple not susceptible to *Verticillium* wilt," Townsend says.

Such a breeding program is a possibile future project at the Arboretum, he adds.

One of the findings that surprised Townsend was how long the fungus itself could survive.

"We were able to find the fungus in the trees more than a year after we inoculated them," he says. "We had expected that the fungus would live only a few months at most and certainly not after a winter."

The ability of the fungus to survive so long suggests breeding a nonsusceptible Norway maple is as important as finding a treatment.—By **J. Kim Kaplan,** ARS.

Alden M. Townsend and Lawrence R. Schreiber are with the U.S. National Arboretum, 24th and R Streets, N.E., Washington, DC (202) 475-4824. ◆

Treatment for Africanized Bee Stings Possible

Developing an antivenom treatment for Africanized bee stings should be feasible, says ARS' Justin O. Schmidt, because the venom is quite similar to that of domestic honey bees.

Entomologist Schmidt and coworkers at the Carl Hayden Bee Research Center in Tucson, Arizona, compared proteins in the venom of more than 1,000 bees of each race and found only minor differences. In fact, antibodies against honey bee venom found in a blood sample from a commercial beekeeper proved useful in counteracting Africanized bee venom.

Despite their fearsome reputation—Africanized bees may have killed as many as 600 people worldwide—individual bees contain less venom than European bees. And this venom is no more toxic either, says Schmidt, who tested it with laboratory mice.

"However, the danger to humans posed by Africanized bees is still very real. These bees are extremely defensive, and unlike European bees who typically attack only a few at a time, Africanized bees can go on an attack frenzy with hundreds storming a victim at the same time," he says.

"I'd compare an attack by 500 bees to roughly the equivalent of a rattlesnake bite. Most persons who are massively envenomed by hundreds of Africanized bee stings would become very ill even with the present treatments designed for European stings," says Schmidt, who cooperated in the studies with University of Arizona allergist Michael J. Schumacher, M.D.—By **Dennis Senft,** ARS.

Justin O. Schmidt is at the USDA-ARS Carl Hayden Bee Research Center, 2000 East Allen Road, Tucson, AZ 85719 (602) 670-6380. ◆

U.S. Department of Agriculture
Agricultural Research Service
Rm. 318, B-005, BARC-West
Beltsville, MD 20705

Bulk Rate
Postage and Fees Paid
U.S. Department of Agriculture
Permit No. G-95

Official Business
Penalty for Private Use—$300

To stop mailing ☐
To change your address ☐
Send mailing label on this magazine and your new address to above address.

☐ Please send me a year of **Agricultural Research** SN 701-006-00000-3

Charge your order. It's easy!

1. The total cost of my order is $24.00. (International customers $30.00.) All prices include regular domestic postage and handling and are good through August 1990. After this date, please call Order and Information Desk at (202) 783-3238 to verify current price.
2. **Please Type or Print**

(Company or personal name)

(Additional address/attention line)

(Street address)

(City, State, ZIP Code)

( )

(Daytime phone including area code)

3. **Please choose method of payment:**

☐ Check payable to the Superintendent of Documents

☐ GPO Deposit Account

☐ VISA or MasterCard Account

(Credit card account number)

Thank you for your order!

(Credit card expiration date)

Signature)

4. **Mail to:** Superintendent of Documents, Government Printing Office, Washington, DC 20402-9325